

Review on Pharmacogenetics and Pharmacogenomics Applied to the Study of Asthma

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Abstract

Nearly one-half of asthmatic patients do not respond to the most common therapies. Evidence suggests that genetic factors may be involved in the heterogeneity in therapeutic response and adverse events to asthma therapies. We focus on the three major classes of asthma medication: β -adrenergic receptor agonist, inhaled corticosteroids, and leukotriene modifiers. Pharmacogenetics and pharmacogenomics studies have identified several candidate genes associated with drug response.

In this chapter, the main pharmacogenetic and pharmacogenomic studies in addition to the future perspectives in personalized medicine will be reviewed. The ideal treatment of asthma would be a tailored approach to health care in which adverse effects are minimized and the therapeutic benefit for an individual asthmatic is maximized leading to a more cost-effective care.

Key words Asthma, β -adrenergic, Corticosteroids genes, Leukotriene, Pharmacogenetics, Pharmacogenomics, Personal medicine

1 Introduction

Asthma is a phenotypically heterogeneous disease caused by interactions among demographic, social, environmental, and genetic factors [1, 2]. The disease is characterized by variable degrees of airflow obstruction and airway inflammation producing breathlessness and wheezing [3]. Also, it is one of the most common chronic diseases currently affecting more than 300 million people worldwide. The annual death rate from complications of asthma approximates to 200,000 people worldwide, mainly in less developed countries. It is estimated that these numbers may be increased by 20% over the next 10 years if adequate measures are not taken [4].

It is difficult to quantify the global economic burden of asthma. The indirect cost of asthma, especially its negative impact on productivity, is very high. According to The Global Asthma Report 2014, the estimated total cost to society in the United States of America

was \$56 billion in 2007, or \$3259 per person per year (in 2009 US dollars). The impact of these indirect costs would be diminished by improving asthma control, through improving access to good management including medicines for controlling the disease (<http://www.globalasthmareport.org/>). The complexity of asthma control lies in the different response pathways of drugs and the variable responses from patients.

Nearly 70–80% of patients with asthma may have high variability in the clinical response to the most common asthma medications. Among main factors that may be responsible for this variability, poor patient adherence, ineffective methods of drug delivery, incorrect drug selection are included; however, in most cases, the interindividual differences cannot be explained only by these factors, suggesting that there may be a genetic component that could play an important role [5].

The Human Genome Project is an international initiative designed to identify the sequence of the human genome. Following with the publication in 2001 of a sequence draft, investigators have focused on genomic variations among individuals [6]. These variations include single nucleotide polymorphisms (SNPs), base insertions or deletions, copy-number variations, and variable numbers of tandem repeats. SNPs are polymorphisms that affect a single base. Some of these variations could affect the quantity, timing, and function of the encoded protein, contributing to the differences in drug response among individuals [7], providing the rationale for pharmacogenetic research.

The term pharmacogenetics was coined by Vogel in 1959 [8]. Pharmacogenetics refers to the study of inherited variations in one single gene on drug response, and pharmacogenomics is the study of the role of inherited and acquired genetic variation in many different genes (genome) including DNA and RNA that determine drug response [9, 10]. The goal of pharmacogenomics is to facilitate the identification of biomarkers that can help optimize drug selection, dose, and treatment duration and prevent adverse reactions. Pharmacogenomics can provide new insight into mechanisms of drug action and contribute to development of new therapeutics [9].

2 Methods for Discovery Pharmacogenetic Variants

The approaches in pharmacogenomic studies have evolved from individual candidate gene studies for evaluation of drug pathways to genome-wide association studies (GWASs) to identify novel variations affecting drug response [10]. In addition, analysis of other data, like expression or biochemical data, may identify gene candidates.

2.1 Studies of Candidate Gene

The candidate gene association studies have been used in many pharmacogenetic studies of asthma. These approaches interrogate whether a particular allele or a set of alleles is more frequent in patients who have a better or worse drug response [11, 12]. Candidate gene studies are relatively cheap and quick to perform. Genes are selected based on their known physiological or pharmacologic effect on disease or drug response. So a previous knowledge about the function of a gene is essential to select the candidate gene to study. This is followed by identification and selecting SNPs having a functional consequence as the function of a protein. Finally, the gene variant is associated to the drug response (trait) by detecting its existence in random test subjects (cases) having the trait and the selected control subjects, which do not [12].

In pharmacogenetic studies, SNPs with minor allele frequencies (MAF) above 0.10 are generally selected for detecting genetic associations. SNPs with MAFs lower than 0.10 are less likely to report a genetic association. An exception occurs when SNPs with low MAFs causes functional effects, such as an alteration in gene expression or in the functional effects on the protein [5]. The principal advantage of this approach is that requires a relative small sample size, is cheap, simpler to perform and to interpret because the physiological or pharmacologic effects of the genes are previously well characterized. The main disadvantage is to require previous knowledge of the function of the gene concerning to drug response. If information about the gene function is limited, the selection of the gene is difficult to support.

2.2 Biological Pathway Studies

This approach analyzes multiple genes encoding several components of a drug response pathway, involving important genes for controlling steps in drug response. The strategy for selecting genetic SNPs is nearly identical to candidate gene studies [5].

2.3 Genome-Wide Association Studies (GWAS)

GWAS allow the analysis of hundreds of thousands of SNPs with the goal of relating them to a specific phenotype, a disease, or a better or worse drug response. In a GWAS, the SNPs representing regions of the genome with the most interindividual variation are probed on a DNA microarray for each individual in a set of cases and controls. Each SNP is tested independently and the significance (p -value) must be corrected for multiple hypotheses, usually using Bonferroni correction [13]. When an SNP reaches the genome-wide significance, it is a candidate for following analysis.

The main advantage of GWASs has been their excellent resolution, their statistical power to detect risk variants with moderate effects, and to be able of analyze the whole genome. On the contrary, GWASs need large sample sizes and tools for processing a very large pool of data. A huge number of statistical comparisons (hundreds of thousands) make that the threshold defining significant associations should be lower than conventional

case-control studies [6, 14]. Like the candidate gene association studies, the results should be replicated in multiple independent populations.

As the price of high-throughput sequencing is decreasing, many researchers are performing exome or whole-genome sequencing to identify genetic factors of drug response. This approach is also hypothesis-independent in the identification of SNPs, detects less common mutations, and captures larger-scale information, including copy-number variants (CNVs) and structural variants [13].

2.4 RNAseq

RNA expression data from microarrays or RNAseq from drug-treated samples could serve as start point in discovering new genes involved in drug response. Using expression profiles generated from a drug-treated sample compared to control can be used to determine a molecular response to a drug. Usually, these experiments are carried out in cell lines that provide a valuable low-cost resource [15].

2.5 Expression Quantitative Trait Loci (eQTL)

eQTL are genomic loci that contribute to variation in expression levels of mRNAs or protein, usually a single gene product with a specific chromosomal location. There are cis-eQTLs that map near the gene and can act locally, and those mapped far even in another chromosome, the trans-eQTL, which can act at a distance (distant eQTLs). The integration of the analysis of DNA and RNA sequencing offers a unique view of the genetic causes of variation in the transcriptome. The variants of eQTL and the consequent variation of expression level can add a functional dimension to our knowledge, helping the understanding of the mechanisms leading this variation [16].

2.6 Pathway Discovery

Once the candidate gene is identified, analyzing genetic networks, cascades, and pathways can help to discover other possible candidates that affect the drug action. The information on the gene's network or pathway could serve to limit or generate new hypotheses in the pharmacogenomics of the drug.

3 Asthma Therapy: Pharmacogenetics and Pharmacogenomics Studies

Currently, the most common therapeutic options for treating asthma include inhaled β_2 -adrenergic receptor agonists (beta agonists), inhaled corticosteroids (ICS), leukotriene modifiers, inhaled anticholinergics, and theophylline. In the near future, biologic therapies will also have an important place in the therapy of asthma. These drugs are classified into two groups according to the prevention of asthma or the symptom relief. The former groups are anti-inflammatory, especially inhaled corticosteroids and long-acting

beta agonists (LABA). The second group includes drugs that act quickly relieving acute bronchoconstriction and symptoms like coughing, chest tightness, and wheezing, as short-acting beta agonists (SABA).

The ideal asthma treatment should be a tailored approach to health care in which adverse effects will be minimized and the therapeutic benefit for the patient is maximized. The clinical application of knowledge of patient's genetic profile can play a main role in this aspect.

3.1 β -Adrenergic Receptor Agonists

Inhaled beta agonists are the most commonly prescribed medical therapies for the management of asthma. Depending on the duration of their action, three types of inhaled beta agonists exist: the short-acting beta agonists (SABA: fenoterol, isoproterenol, pirbuterol, levalbuterol, and albuterol), the long-acting beta agonists (LABA: salmeterol and formoterol), and the ultra-long-acting beta agonists (vilanterol and indacaterol). The LABA therapy is administered in combination with an ICS as regular controller therapy, while SABA therapy is used for rescue, as-needed treatment for acute symptom relief or the prevention of exercise-induced symptoms. Despite the common use of these agents, this drug class is the center of a controversy related to concerns over adverse events. High doses of SABAs with less selective beta-adrenergic receptor activity were associated with serious adverse effects (including death) [17]. These drugs exert their action by activation of β_2 -adrenoreceptors (*ADRB2*) abundantly expressed in bronchial smooth muscle cells. The resulting of this activation leads to muscle relaxation, airway dilatation, and improved airflow [18]. In Table 1, genetic variants associated with response to β -adrenergic receptor agonist are shown.

3.1.1 Candidate Gene Analyses and Pathway Studies

ADRB2

The β_2 -adrenergic receptor (beta2-AR) is a member of the G-protein-coupled adrenergic receptor family with seven transmembrane segments; it was cloned by Kobilka et al. in 1987 and is localized to chromosome 5q31–q32, a region that has been linked with asthma and asthma-related phenotypes [19]. The *ADRB2* gene has been resequenced in multiple populations and more than 80 SNPs have been identified [20]. Two of these SNPs, Arg16Gly (rs1042713) and Glu27Gln (rs1042714), are the most common and have been well characterized in asthma pharmacogenetics [21]. The estimated frequency of the Arg16 variant is 39.3% in white Americans, 49.2% in blacks Americans, and 51.0% among Chinese [22].

With respect to correlation between this SNP and SABA, several studies demonstrated that Arg16 homozygotes had a greater acute response to SABA bronchodilation compared with Gly16 homozygotes, which has been confirmed in additional asthma populations [23, 24]. Several studies demonstrated higher FEV1

Table 1
Genetic variants associated with response to β -adrenergic receptor agonist

Gene	RsID	Study design	Drug class	Clinical association
<i>ADRB2</i>	rs1042713	Candidate gene	SABA	FEV1 bronchodilation PEFR response PEFR response
	rs1800888	Candidate gene	LABA LABA	PEFR response Bronchoprotection Exacerbation asthma
<i>ADCY9</i>	rs2230739	Candidate gene	SABA LABA	FEV1 response
<i>GSNOR</i>	Promoter region	Candidate gene	Albuterol	Broncodilatador response
<i>ARG1</i>	rs2781659 rs2781667	Candidate gene	SABA	FEV1 bronchodilation
<i>ARG2</i>	rs7140310 rs10483801	Candidate gene	SABA	FEV1 bronchodilation
<i>NOS3</i>	rs1799983	Candidate gene	SABA	FEV1 bronchodilation
<i>SPATS2L</i>	rs295137	GWAS	SABA	FEV1 bronchodilation
<i>SLC22A15</i>	rs1281748 rs1281743	GWAS	SABA	FEV1 bronchodilation
<i>SLC24A4</i>	rs77441273	GWAS	SABA	FEV1 bronchodilation

(forced expiratory volume in the first second) increases after SABA (salbutamol) administration in homozygous Arg16 individuals as compared to heterozygous and homozygous Gly16 patients with polynosis [25, 26]. Patients who were homozygous Arg16 had impaired SABA response compared to homozygous Gly16 individuals [27]. Systematic administration of SABA to Arg16 asthmatics caused deterioration of lung function that did not stop even with treatment discontinuation. In contrast, patients homozygous for Gly16 demonstrated improved lung function [28, 29].

Some patients treated with salmeterol, experienced rare but severe asthma exacerbations [30]. Further investigation suggested dependence between Arg16 genotype and faster decline of lung parameters (FEV1) after LABA application [31]. However, certain controversy regarding adverse events has been reported [32, 33].

Regarding the relationship of this genetic variation in response to treatment in the pediatric population, it has been reported that Arg16 genotype increased susceptibility to exacerbations in children with asthma on regular LABA therapy. Montelukast may be proposed as tailored second-line controller therapy instead of salmeterol in asthmatic children [34]. The Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory Effects (PACMAN) cohort study concluded that Arg16 homozygotes have a poorer response when treated with LABA and ICS [35]. This SNP at position 16 appears not to have clinical relevance to justify genotyping individuals [10].

In addition to these common SNPs, other less common, non-synonymous coding variants have also been reported in the *ADRB2* gene. For example, the SNP rs1800888 encodes a Threonine to Isoleucine substitution at amino acid position 164 (Thr164Ile). The Ile164 isoform is three-to-four times less responsive to agonist-induced stimulation than carriers of the wild-type Thr164 [36]. Ortega et al. reported that Ile164 and -376ins were associated with adverse events during LABA therapy [37].

ADCY9

Adenylyl cyclase (*ADCY9*) belongs to the G-protein coupled β 2-adrenergic receptor pathway. Polymorphisms in the gene encoding of this enzyme have been analyzed in several studies. Tantsira et al. [38] performed a study, in that SNP Ile772Met (rs2230739 in *ADCY9*) was associated with acute bronchodilation in response to SABA in ICS-treated asthmatics from the CAMP study, while Kim et al. found that this SNP was also associated with lung function response to a LABA and ICS treatment in a Korean asthma population [39]. On the other hand, new studies conducted by Drake et al. have also shown that rare variants adjacent to *ADCY9* and *CRHR2* are associated with albuterol bronchodilator response in Puerto Rican and Mexican asthma subjects from the GALA (Genetics in Latino Americans) cohort [40].

GSNOR

Nitric oxide bioactivity, mediated through the formation of S-nitrosothiols (SNOs), has a significant effect on bronchomotor tone. S-nitrosoglutathione reductase (*GSNOR*) metabolizes S-nitrosothiol (SNO), an endogenous bronchodilator that is decreased in children with asthmatic respiratory failure and in adults with asthma undergoing segmental airway challenge [41]. Choudhry et al. recently sequenced the *GSNOR* gene in a subset asthmatics cohort and identified 13 SNPs with an allele frequency greater than 5% [42]. Association studies identified gene-gene interactions between *GSNOR* and *ADRB2* in Mexicans and Puerto Ricans combined, although these SNPs and their haplotypes were not associated with bronchodilator response.

ARG1 and *ARG2*

The arginases (*ARG1* and *ARG2*) metabolize L-arginine, a natural substrate for nitric oxide synthase (NOS), to generate nitric oxide, which is an endogenous bronchodilator. Several recent studies support the role of *ARG1* and *ARG2* polymorphisms in bronchodilator response (BDR). Litonjua et al. reported that *ARG1* polymorphisms (rs2781659 and rs2781667) were associated with an acute SABA bronchodilator response in asthmatics [43]. These results have been replicated in independent asthma trial cohorts [44]. Vonk et al. conducted a study in a Dutch asthma cohort in that two polymorphisms in *ARG2* (rs17249437 and rs3742879) were associated with asthma and with more severe airway obstruction [45].

NOS3 Nitric oxide synthase enzymes have an important role in airway inflammation in asthmatic children. Iordanidou et al. investigated the association between NOS gene polymorphisms and response to ICS and LABA combination therapy demonstrating that the polymorphism Asp298Glu (rs1799983 in *NOS3*) was associated with lung function response to LABA and ICS [46].

3.1.2 GWASs Studies

SPATS2L

Himes et al. identified an SNP rs295137 close to the *SPATS2L* gene. Subsequently it has been suggested that *SPATS2L* may be a relevant regulator of β 2-adrenergic receptor down regulation [47]. Another GWAS of BDR identified 4 SNPs (rs350729, rs1840321, rs1384918, and rs1319797) associated with BDR in chromosome 2 [48]. An intergenic SNP (rs11252394) was identified in the Childhood Asthma Management Program (CAMP) cohort-mediated GWAS of acute BDR to inhaled albuterol. This SNP is located proximal to several excellent biological candidates including protein kinase C (*PRKCQ*), interleukin receptors (*IL15RA*, *IL2RA*) among others [49].

SPATA13-AS1

Padhukasahasram et al. significantly associated *SPATA13-AS1* with SABA-induced bronchodilatation in healthy African Americans and replicated in two African American cohorts and one European American cohort with asthma [50].

SLC22A15

Drake KA et al. performed a GWAS with the objective of identifying genetic variation associated with BDR. Seven genetic variants associated with BDR were identified with significant threshold. Two rare variants were also identified in *SLC22A15* gene, being associated with increased BDR in Mexicans [40].

3.2 Inhaled Corticosteroids (ICS)

As previously commented, there are a group of patients less responsive to ICS, in which pharmacogenetic approaches might lead to improved personalized medicine [51]. The combination of wide interindividual variability and high intraindividual repeatability supports a genetic difference for the response to ICS in asthma [52]. In Table 2, genetic variants associated with response to inhaled corticosteroids are shown.

3.2.1 Candidate Gene Analyses and Pathway Studies

NR3C1

One of the earliest pharmacogenetic studies investigating glucocorticoids response involved the glucocorticoids receptor gene (*NR3C1*) located in chromosome 5q31. Two SNPs have demonstrated potential effects on corticosteroid response. The first is a relatively rare coding mutation, Asn363Ser (rs56149945 in *NR3C1*), which has been identified in several populations. Huizenga et al. conducted a study in which it was observed that lymphocytes from individuals with receptors with this genetic variation were shown to have a greater sensitivity to dexamethasone compared to lymphocytes from noncarriers [53].

Table 2
Genetic variants associated with response to inhaled corticosteroids

Gene	rs ID	Study design	Clinical association
<i>NR3C1</i>	rs56149945	Candidate gene	Sensitivity to drug
<i>CRHR1</i>	rs242941 rs1876828 rs242939	Candidate gene	Lung function FEV1 response
<i>TBX21</i>	rs2240017	Candidate gene	Bronchoprotection
<i>STIP1</i>	rs6591838 rs1011219	Candidate gene	FEV1 response
<i>GLCCI1</i>	rs37972	GWAS	FEV1 response
<i>T</i> gene	rs3127412 rs6456042	GWAS	FEV1 response
<i>FBXL7</i>	rs10044254	GWAS	ICS response
<i>CA10</i>	rs967676	GWAS	ICS response
<i>SGK493</i>	rs1440095	GWAS	ICS response
<i>CTNNA3</i>	rs1786929	GWAS	ICS response

The other genetic variation has potential pharmacogenetic implications. Gene expression studies have shown that the presence of the G allele in this mRNA stability motif increases the stability of GR β mRNA, thus allowing increased rates of GR β translation. Asthmatics possessing this G allele could therefore produce more GR β , thus competing with functional GR α in the steroid dimer complex. These individuals could also have an attenuated response to exogenous and endogenous corticosteroids [5].

CRHR1

The *CRHR1* gene is the major receptor for corticotrophin, and it is the key regulator of corticosteroids synthesis and catecholamine production. The results of pharmacogenetic studies in this gene have been very promising. The most important pharmacogenetic studies have been conducted by Tantisira et al. [54]. This study found a significant correlation between lung function improvement after inhaled corticosteroid therapy and SNPs (rs1876828, rs242939, and rs242941). Homozygous individuals with this polymorphism had average FEV1 improvement, higher than homozygous patients lacking this SNP.

TBX21

Another candidate gene involved in ICS response is *TBX21*. *TBX21* knockout mice develop bronchial hyperresponsiveness, enhanced airway eosinophilia, and faster airway remodeling proving. The SNP His33Glu (rs2240017) has been associated with improvements in bronchial hyperresponsiveness or bronchoprotection. Tantisira et al. [55] conducted a study in the CAMP cohort

that showed a significant decrease in airway hyperresponsiveness in heterozygous subjects for this SNP during ICS treatment as compared to His33His homozygous subjects and individuals that are not ICS-treated. This study was replicated in an independent Korean cohort with similar results [56].

FCER2

The *FCER2* gene encodes the low-affinity receptor for IgE, a key molecule for B-cell activation and growth. A novel variant in *FCER2* has recently been associated with asthma exacerbations while on ICS. The SNP, rs28364072, was associated with increased risk of exacerbations in asthmatic children taking ICS [57]. This novel variant was also associated with both higher IgE levels and with differential expression of the *FCER2* gene, supporting the contention that variation in *FCER2* can adversely affect normal negative feedback in the control of IgE synthesis and action. Others studies have been also confirmed these results [58, 59].

STIP1

Multiple genes encode for the heterocomplex of chaperones and immunophilins that bind the glucocorticoids receptor and mediate proper assembly and activation of the receptor. Genetic variations in the gene encoding for the heat shock organizing protein (*STIP1*) have been associated with the regulation of corticosteroid response in asthmatic subjects with reduced lung function. Thus, Hawkins et al. found that the SNPs (rs6591838, rs2236647, and rs1011219 in *STIP1*) were significantly associated with improvement in FEV1 response after 4 or 8 weeks of corticosteroid therapy [60].

3.2.2 GWASs Studies

Investigations into the genetic basis for treatment response heterogeneity in asthma have largely focused on candidate genes. These candidate gene pharmacogenetic investigations have identified associations related to the variable response to corticosteroids as measured by differences in lung function, airways responsiveness, bronchodilator response, and exacerbations. However, progress related to identifying sufficient numbers of variants to achieve the predictive medicine for any ICS treatment phenotype through the use of candidate genes has been slow. GWAS provides the ability to rapidly identify novel pharmacogenetic variants by simultaneously interrogating genetic variants from across the genome. The following describe the most important GWASs studies to date:

GLCCI1

A recent study demonstrated an SNP in the promoter region of the glucocorticoids-induced transcript 1 gene (*GLCCI1*), rs37972, which was associated with lung function responses to inhaled glucocorticoids. The SNP rs37972 is also in strong linkage equilibrium (i.e., strongly correlated or “tagged”) with another *GLCCI1* promoter SNP, rs37973, which determines gene transcription in vitro, demonstrating a functional or molecular-based rationale for

the observed genetic effects of variation in this gene on corticosteroid response. It will be important to replicate these corticosteroid response gene variants in other, larger populations and determine whether they are independent predictors or have additive effects that regulate corticosteroid responses in asthma [61].

T Gene

Another GWAS using the SHARP cohort identified variants in the *T* gene (*T*, brachyury homolog) (rs3127412, rs6456042) [62].

FBXL7

Park et al. performed the first GWAS of ICS response. Three SNPs were found but were not replicated in adult cohorts; rs10044254 was found in intronic region of *FBXL7* gene and is associated with decreased expression in immortalized B cell derived from CAMP participants. Author suggested that specific mechanism regulating symptomatic response to ICSs in children does not work in adults [63].

CA10, *SGK493*, and *CTNNA3*

Perin and Potěnick have performed a GWAS in different phenotypes of childhood asthma. Authors reported significant associations between *CA10* (rs967676), *SGK493* (rs1440095), and *CTNNA3* (rs1786929) with asthma and glucocorticoids response [64].

Wang et al. identified rs6924808, rs10481450, rs1353649, rs12438740, and rs2230155 significantly associated with corticosteroid dose-response. This report is the first and unique with this pharmacodynamic approach, and has been revealed to be statistically more powerful for gene detection compared with classical single-dose approaches [65].

Himes et al. performed an RNAseq analysis to characterize transcriptomic changes in four primary human airway smooth muscle (ASM) cells in response to dexamethasone (potent synthetic glucocorticoids). Authors identified *CRISPLD2*, which encodes a secreted protein previously implicated in lung development and endotoxin regulation. It was found to have SNPs associated with inhaled corticosteroid resistance and bronchodilator response among asthma patients. Quantitative RT-PCR and Western blotting showed that dexamethasone treatment significantly increased *CRISPLD2* mRNA and protein expression in ASM cells. Functional studies revealed that *CRISPLD2* is an asthma pharmacogenetics candidate gene that regulates anti-inflammatory effects of glucocorticoids in the ASM [15].

Another integrative approach combines an eQTL analysis with GWAS data and expression microarrays from lymphoblastoid cell lines in corticosteroid treated and untreated cells. Multiple novel genome-wide significant pharmacogenomic loci were identified in both Caucasian and African Americans including rs6504666 and rs1380657 (*SPATA20*), rs12891009 (*ACOT4*), rs2037925 and rs2836987 (*BRWD1*), rs1144764 (*ALG8*), and rs3793371 (*NAPRT1*) [66].

Table 3
Genetic variants associated with response to Montelukast

Gene	rs ID	Study design	Clinical associations
<i>ALOX5</i>	Promoter repeat rs892690 rs2029253 rs2115819	Candidate gene	Changes FEV1 and PEF Exacerbation risk
<i>LTC4S</i>	rs730012	Candidate-gene	Exacerbation risk
<i>CYSLTR2</i>	Rs912278	Candidate gene	Changes in PEF
<i>ABCC1</i>	rs119774	Candidate gene	Changes in FEV1
<i>LTA4H</i>	rs2660845	Candidate gene	Decrease of FEV1 and PEF
<i>SLCO2B1</i>	rs12422149	Candidate gene	Decrease of serum drug levels
<i>MRPP3</i>	rs12436663	GWAS	Decrease of FEV1
<i>GLT1D1</i>	rs517020	GWAS	Poor response
<i>MLLT3</i>	rs6475448	GWAS	Improve response

**3.3 Leukotriene
Modifiers**

Leukotriene modifiers play an important role in a variety of inflammatory diseases such as asthma [58]. This drug class shows strong anti-inflammatory activity, ameliorate asthma clinical course and improve disease control with minimal or no side effects. Currently, based on their mechanism of action, there are two groups of drugs: cysteine leukotrienes receptor antagonists (montelukast, zafirlukast, pranlukast, and tomelukast) and 5-lipoxygenase inhibitors (zileuton). In Table 3, genetic variants associated with response to leukotriene modifiers are shown.

**3.3.1 Genes Candidates
Analyses and Pathway
Studies**

To date, most pharmacogenetics investigations, which may affect therapy with leukotriene modifiers, have focused on variants of *ALOX5* and *LTC4S*. Other genetic variants of *LTA4H*, *MRP1*, and *CYSLTR1* have also been investigated.

ALOX5

The *5-LOX* gene (*ALOX5*) is located on chromosome 10q11.12, contains 14 exons, and its activity is associated with a number of repetitions of Sp1/Erg1 binding motifs in the promoter region. Mutant *ALOX5* repeat polymorphism was associated with decreased exacerbation rates in white asthmatic patients treated with montelukast [67], while other studies show a decrease number of asthma exacerbations, improvement of forced expiratory volume at 1 s (FEV1), and decreased use of beta2 agonists in Spanish patients with wild-type allele or heterozygous variant allele [68] or no significant difference in terms of bronchodilator response or bronchial hyperresponsiveness between wild-type allele and heterozygous variant allele [69].

In a recent genetic study of the *ALOX5* promoter, variant in homozygotes for the less common repeat alleles had a higher urinary concentration of leukotriene E4, consistent with increased leukotriene biosynthesis. Most importantly, 86% of these minor risk alleles were from African American subjects suggesting that genetic variation from an African ancestry may influence asthma severity and responsiveness to therapies targeting the leukotriene pathway [70]. Subsequent larger candidate gene studies have shown that additional *ALOX5* SNPs (rs2115819, rs4987105, and rs4986832) may also influence the response to the leukotriene receptor antagonist, montelukast [67, 71].

LTC4S

LTC4 synthase (*LTC4S*) belongs to S-glutathione synthases family and catalyzes the conversion of LTA4 to LTC4. The most important polymorphism detected is the A-444C (rs730012), which is implicated in an increase in the production of LTC4 in eosinophils. Sanak et al. confirm that this genetic variant occurs more often in patients with aspirin idiosyncrasy [72]. While Lima et al. found that in heterozygous individuals for this variation had a 73% lower risk of asthma exacerbations compared to homozygous individuals during the treatment with montelukast [67]. Other studies performed in Southern Europe [73] and Japan [74] also reported similar results.

CYSLTR1 and CYSLTR2

The *CYSLTR1* and *CYSLTR2* receptors have been characterized as G-protein-coupled receptors. Some studies have examined the relationship of polymorphisms in these genes with leukotriene modifiers, but these results are not very promising. Tansira et al. conducted a study on patients treated with zileuton, but not significant correlation could be established between *CYSLTR1* gene polymorphisms and clinical response to therapy [75]. However, Klotsmant et al. performed a study in which a strongest statistical evidence of clinically relevant pharmacogenetic effects peak expiratory flow was identified in *CYSLTR2* (rs91227 and rs912278) [71].

ABCC1

The *ABCC1* gene encodes MRP1 (Multiple Drug Resistance Protein 1), which plays an important role in transmembrane transport of LTC4. One of the polymorphisms of this gene (rs119774 in *LTC4*) had been correlated to montelukast response. Lima et al. observed that in heterozygous individuals for this polymorphism had a 24% FEV1 rise as compared to only a 2% improvement in homozygous individuals [67].

LTA4

LTA4 hydrolase is an enzyme that converts LTA4 to LTB4. The gene encoding this protein is located on chromosome 12q22. One of the known polymorphisms for this gene (rs2660845 in *LTA4*) has been associated with risk of asthma exacerbation.

Heterozygous individuals for this SNP have 4–5 higher risk of asthma exacerbation than homozygous individuals, during treatment with montelukast. The pathogenic mechanism of this process remains unclear. It has been hypothesized that this SNP causes a decreased enzyme activity that results in diminished LTB₄ synthesis, therefore stimulating the LTC₄-synthase pathway and leading to cysteine leukotriene synthesis [67].

SLCO2B1

The gene *SLCO2B1* encodes the protein 2B1 that plays an important role in the active transport of organic anions through the intestinal wall. *SLCO2B1* gene polymorphisms have been studied in order to establish their relationship with montelukast pharmacokinetics. The most important polymorphism described is Arg312Gln (rs12422149), which is involved in the transport and serum levels of montelukast. Mougey et al. demonstrated that individuals with this SNP have a significantly lower serum drug concentration [76]. However, these effects of polymorphisms of the *SLCO2B1* transporter gene on the pharmacokinetics of montelukast have not been reproduced in smaller studies [77, 78].

3.3.2 GWAS Studies

The first GWAS of the leukotriene modifier response in asthma has been recently published. The study analyzed DNA and phenotypic information from two placebo-controlled trials of zileuton response evaluating change in FEV₁ following leukotriene modifiers treatment. The top 50 SNP associations were replicated in an independent zileuton cohort, and two cohorts of montelukast response. The rs12436663 in *MRPP3* was significantly associated. The homozygous carriers showed a significant reduction in FEV₁ change following zileuton treatment. The rs517020 in *GLT1D1* was associated with poor response after montelukast and zileuton treatment [79]. A second GWAS was performed using phenotypic data available from American Lung Association-Asthma Clinical Research Center (ALA-ACRC) cohorts. A novel pharmacogenomic locus (rs6475448) in *MLLT3* was identified associated to improved montelukast response in asthmatics [80].

4 Future Perspectives

In last years, human genetic research has made a considerable progress in both genome analysis techniques and international research networks for human genome information (consortiums). Pharmacogenetics has evolved into pharmacogenomics by the application of GWASs, next-generation sequencing, and newer integrative techniques to study drug response phenotypes. Large-scale whole-exome and whole-genome sequencing projects such as the National Institutes of Health National Heart, Lung, and Blood Institute GO Exome Sequencing Program, the 1000 Genomes

Project and the Consortium of Asthma in African Ancestry Populations have resulted in an excellent database of common and rare genetic variants, for future pharmacogenetic studies in different racial and ethnic groups [81, 82].

Many current studies are limited by their small sample size, imprecise phenotype definitions, population stratification, and lack of replication of the results. Future studies should focus on these limitations. However, other factors, such as gene-environment interactions, epigenetic regulation, and interactions between variants in different genes and genetic pathways, DNA methylation, Histone modifications, transcriptional regulation by small interfering RNA (siRNA), micro RNA (miRNA), and long non-coding RNA (lncRNA), are also being explored as biomarkers in therapeutics interventions [83–85]. In addition to molecular genetics studies, functional studies in cellular models or animals as shown in this book are also necessary for studying variation in the expression of the different polymorphic genes. Another strategy for drug development is a biological system approach, focusing in multiple omics like metabolomics and pharmaco-proteomics studies of an animal model [10]. Translating the findings into clinical practice is the main challenge to scientific. Some of the identified genetic variations have large effects and predict drug responses. These pharmacogenomics biomarkers may determine which patients will benefit from a specific drug. The cost of high-throughput technologies are decreasing very fast and it is expected that in near future, these should be proposed for use in clinical trials and this user-friendly technology should help to test individual patients.

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